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Detection and Genetic Characterization of Sporadic Noroviruses in Nara Prefecture between April 2006 and September 2007

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Norovirus (NoV) is usually prevalent in the early winter season and causes symptoms of acute gastroenteritis such as diarrhea or vomiting upon infection. According to a surveillance report by the Forborne Viruses in Europe Network, two new GII/4 variants (E2006a and E2006b) appeared during the 2005/2006 season, and these variants were globally observed (1,2). Siebenga et al. reported in detail the genetic changes in NoV GII/4 capsid proteins from 1995 to 2006 (3). From November to December in 2006 in Japan, extensive outbreaks of gastroenteritis caused by NoV GII/4 strains were recorded in hospitals, hotels, schools, nursing homes, and other sites. To clarify the genetic characteristics of sporadic NoV in Nara Prefecture, Japan, from April 2006 to September 2007, we phylogenetically analyzed nucleotide sequences in regions of the ORF2 obtained from surveillance specimens.

Between April 2006 and September 2007, a total of 289 stool specimens were collected from patients with acute gastroenteritis in Nara Prefecture. Viral RNA was extracted using the RNeasy Mini Kit (Qiagen, Valencia, Calif., USA), and then reverse transcription was performed with ReverTra Ace reverse transcriptase (Toyoobo, Osaka, Japan). PCR was performed with the COG1/2F and SKG1/2R primer pair. The NoV GI genotype was detected in 84 (29.0%) of these specimens, and no NoV GI-positive specimens were detected. The 74 NoV GII-positive specimens selected were sequenced using a sequencing kit (Thermo Sequenase Cy5.5 Dye Terminator Cycle Sequencing Kit; GE Healthcare UK, Ltd., Buckinghamshire, UK). Based on these results, a BLAST search was carried out, and then phylogenetic analysis was performed using the neighbor-joining method with the Kimura two-parameter model. For the phylogenetic analysis of the capsid sequences (282 bp), sequences from GenBank were included, and the GenBank accession numbers for the reference strains are: X76716 (Bristol), AJ004864 (Grimsby), AY502023 (Farmington Hills), DQ078794 (Hunter284E), EF126964 (Terneuzen70), EF126966 (Nijmegen115), AB291542 (Kobe034), AB220925 (Chiba), AB240187 (Hokkaido), and DQ095875 (Nagano).

The distribution of NoV-associated gastroenteritis patients during April 2006 and September 2007 are shown in Fig. 1. According to our surveillance data, the number of NoV-patients reached a peak in November 2006 (43/84, 51.1%), unlike previous years. A similar pattern was observed in NoV-associated gastroenteritis outbreaks due to food contamination in hospitals, hotels, and schools. In the 2005/2006 season, a peak of NoV activity was observed in early-January (data

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Between April and August 2006, eight NoV GII cases were detected, and classified into four groups as follows: there were 5 cases of GII/2, 1 case of GII/3, 1 case of GII/4, and 1 case of GII/6. A BLAST search revealed that these strains shared high homology (>98.0% nucleotide homology) with MK04/2004/JP (DQ456824), Maizuru/7179/2005/JP (EF028232), Osaka/137/05/JP (AB248844), and Chiba/04-974/2004/JP (AB220925) strains, respectively. Between October 2006 and September 2007, 62 of the 66 (93.9%) NoV-associated cases of gastroenteritis were due to infection with the new GII/4 variant, which has high homology with the Nijmegen115/2006/NL (EF126966), Kobe034/2006/JP (AB291542), and Temeuzen70/2006/NL (EF126964) sequences. These 62 GII/4 cases were phylogenetically analyzed based on the partial nucleotide sequences of the capsid region. As shown in Fig. 2, 56 cases were classified as GII/4-E2006b and 6 cases were classified as GII/4-E2006a strains (90.3 and 9.6%, respectively). Interestingly, since November 2006, this E2006b variant was the most dominant, i.e., it was detected in more than 90% of the GII/4-associated gastroenteritis cases. Moreover, Okada et al. reported that three genetically distant NoV GII/4 subtypes were observed in Chiba Prefecture between October and December 2006 (4). These results demonstrate that the GII/4 genotype evolved between the 2005/2006 and 2006/2007 seasons. Our findings indicate the necessity of careful surveillance for gastroenteritis caused by the new GII/4 variant.

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